Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Contribution of poses screen preimpregnated (PSP) installed at openings and eaves of dwellings in the reduction of malaria transmission in the commune of aguégués in bénin

F. Modeste Gouissi[1*], Sahidou Salifou[2], A. Patrick Edorh[3], A. Rufine Sedjame[1], S.G. Augustin Gouissi[4], W. Anges Yadouleton[5], Martin Akogbeto[5], Michel Boko[1]

[1]*Laboratory of Toxicology and Environmental Health, CIFRED, University of Abomey- Calavi (UAC), 03 BP 1463 Jericho, Cotonou, Benin*
[2]*Polytechnic School of Abomey-Calavi (EPAC), Department of Animal Production, University of Abomey-Calavi (UAC), 01 BP 2009 Cotonou, Benin*
[3]*Department of Biochemistry and Cell Biology, Faculty of Science and Technology (FAST), University of Abomey-Calavi (UAC), 01 BP 526, Cotonou, Benin*
[4]*Faculty of Health Sciences (FSS), University of Abomey-Calavi (UAC), 01 BP 188 Cotonou, Benin*
[5]*Entomological Research Center of Cotonou (CREC), 06 BP 2604 Cotonou, Benin*

ARTICLE INFO

Article history:
Received 10 January 2012
Received in revised form 15 March 2012
Accepted 15 May 2012
Available online 20 January 2013

Keywords:
Malaria
Poses screen pre-impregnated
Anopheles
Hemoglobin

ABSTRACT

Objective: To evaluate the contribution of poses screen pre-impregnated (PSP) installed at openings and eaves of dwellings in the reduction of malaria transmission in the commune of Aguégués in Bénin. **Methods:** The PSP were manufactured from preimpregnated Olyset Net. They were installed at windows, eaves and doors of 70 dwellings. 320 children aged 6-59 months were treated and 311 children were recruited in the control zone. Variables measured are: plasmodic index (IP), gametocyte index, parasite density (PD), fever, hemoglobin, anemia. **Results:** The global IP was 16.62% with PSP and 72.20% without PSP. Gametocyte index did not differ significantly between the treated zone (27.8) and the control zone (29.1). The total geometric mean of DP was 309 in the treated zone and 600 in the control zone. Hemoglobin level is 8.7 in the control zone and 9.5 in the treated zone. We noted a predominance of anemia in the control zone compared to the treated zone. **Conclusions:** The PSP have contributed to a significant reduction in morbidity in the commune of Aguégués.

1. Introduction

Malaria is a common and severe infection caused by five species of the *Plasmodium* genus: *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*), *Plasmodium malariae* (*P. malariae*) and *Plasmodium knowlesi* (*P. knowlesi*). Infections with *P. falciparum* are associated to highest mortality rates, and infections with *P. vivax* and *P. oval* have quiescent liver stages that can lead to subsequent relapses. *P. knowlesi* is a species of malaria which is more and more reported in Asia South East and is distinguished by the fact that primates are the reservoir[1,2]. All kinds of malaria are transmitted by the bites of female anopheles mosquitoes infected. It is rare that the transmission is via blood[3], by exchanging contaminated needles or vertically from mother to fetus[4]. According to the World Health Organization[5], 3.2 billion of people living in 107 countries and territories in 2005 were at a risk of malaria, which resulted in approximately 500 million cases and 1 to 3 million death[6]. Approximately 60% of cases of malaria occur in Africa, 40% in Asia and less than 5% in America. Particularly for malaria with *P. falciparum*, the estimated distribution by region is as follows: 74% in Africa, 25% in Asia and 1% in America[5]. Considering the behavior of malaria vectors (usually active at night), the proper use of treated bed nets (ITNs) is a measure of essential individual protection of malaria control[7,8] and wearing clothes impregnated with insecticide (permethrin) should be considered[9]. The use of insecticide treated mosquito nets greatly increases the protection provided by the net[7,9]. In addition, the insecticide-treated nets for long duration of action are an option to consider[10]. Treated nets represent

*Corresponding author: Gouissi F. Modeste, Laboratory of Toxicology and Environmental Health, CIFRED, University of Abomey- Calavi (UAC), 03 BP 1463 Jericho, Cotonou, Benin.
Tel: 00(229) 97534735
E-mail: CIFRED/ gouissi@yahoo.fr

the vector control tool most promising in the prevention of malaria in Africa south of sahara[9]. In this study, we are led to evaluate the contribution of poses screen pre-impregnated installed at openings and eaves of dwellings in the reduction of malaria transmission in the commune of Aguégués in Bénin.

2. Materials and methods

2.1. Study site

The commune of Aguégués is located at 6 km from the city of Porto-Novo, the administrative capital and 15 km from Cotonou, Benin's economic capital. The municipality covers a surface area of 52 km^2 with 26 650 inhabitants distributed in 21 villages. The habitat, traditional type is made of bamboo built on pilings with a tin roof or straw. The windows and doors of dwellings are small.

2.2. Methods

The study was conducted from May 2011 to September 2011, period of rains. The poses screen preimpregnated are made in the commune by dressmakers using preimpregnated Olyset Nets that we bought at the pharmacy. The study is transversal and has based on children from 6 months to 59 months living in the commune. 320 children from the previous study were randomly selected according the following criteria: (parasitaemia $\geqslant$ 2 500 trophozoites/μL and temperature $\geqslant$ 37.7 ℃): 5%. The investigation of the residence of the children enumerated 70 rooms in 4 villages of the commune. We selected 4 control villages with 311 children whose rooms did not receive the poses screen preimpregnated. Thus, we summarize the villages that received the poses screen preimpregnated in treated zone and untreated villages in control zone. The poses screen preimpregnated are installed in April, one month before the start of the study.

2.2.1. Variables studied

Plasmodic Index (IP) is expressed as a percentage of subjects with Plasmodium in their blood at any stage of development.

Plasmodic Index is about the percentage of carriers of Plasmodium among the subjects examined.

Gametocyte Index (IG) expresses the percentage of subjects whose slide reveals the presence of gametocytes.

Parasitic Density (DP) is estimated from the asexual stages of *P. falciparum* (trophozoites). It is expressed in number of trophozoites of *P. falciparum* per microliter of blood.

Fever: we took the threshold of 37.5 ℃ axillary temperature, from which the child is considered a fever.

Hemoglobin level is expressed in grams per deciliter (g/dL) of blood, which allows to appreciate the state of anemia.

Anemia: The normal value of hemoglobin levels in children less than 6 years according to WHO[11] was set at 11 g/dL of blood.

Anemia is moderate if hemoglobin level is between 7 and 11 g/dL and severe if it drops below 7 g/dL) [12].

2.2.2. Laboratory tests

2.2.2.1. Parasitic research

The thick drop is the technique used. It has the advantage of giving a greater concentration of parasites, which is indispensable when the parasitaemia is minimal. Sampling is done to the pulp of a finger, either at the ear or venipuncture in folds of the elbow. For staining slides, we used the 3% Giemsa for 45 min. The reading is made at the objective 100. The research on parasites and counting are done on thick drop and the identification of different species of plasmodium is done on the smear. Parasite density was determined in 100 microscopic fields, corresponding to 0.25 μL of blood. The number of parasites per microliter of blood or the parasite density is obtained by multiplying the number of parasites in 100 fields by 4.

2.2.2.2. Dosage of hemoglobin level

We practiced the technique of direct reading of the absorbance of the eluates of blood at 550 nm which consists to collect capillary blood on filter paper.

2.2.3. Data analysis

The software Statistica 6 was used to analyze the data. For children, we considered the identification number, location of residence, age, sex, auxiliary temperature, parasite species and the progressive stage, the parasite density and hemoglobin level. The comparison of results was made between control zone and treated zone because the poses screen were administered by zone. Within statistical analysis, we used *Chi*-square test (χ^2) and *P* value for the comparison of categorical variables. The estimates risk of fever, the fraction of fever attributable to malaria, the sensitivity, the specificity were evaluate by the method of Smith *et al*[13]. We divided our sample into six age groups. Thus the following groups were used: 6-11, 12-17, 18-23, 24-35, 36-47, 48-59 (in months).

3. Results

3.1. Distribution of children by sex and age groups

From a total of 631 children aged 6 to 59 months selected for the study, 320 were in the treated zone and 311 in the control zone. Table 1 summarizes the distribution of children by gender, control zone and treated zone.

Table 1
Distribution of children by sex in the control zone and in the treated zone.

Sex	Treated zone		Control zone		Total
	Number	%	Number	%	
Male	122	38.12	120	38.58	242
Female	198	61.88	191	61.42	398
Total	320	100.00	311	100.00	631

Table 2
Distribution of children by age group according to the control zone and the treated zone.

Age (months)	Treated zone		Control zone		Total
	Number	%	Number	%	
6–11	50	15.62	50	16.07	100
12–17	58	18.12	50	16.07	108
18–23	42	13.12	35	11.25	77
24–35	56	17.50	48	15.43	104
36–47	51	15.93	70	22.50	121
48–59	63	19.68	58	18.54	121
Total	320	100.00	311	100.00	631

3.2. Impact of poses screen pre-impregnated (PSP) on parasitological parameters

3.2.1. Impact on plasmodic index, on prevalence of P. falciparum

The impact of the intervention on plasmodic index (IP) in children aged 6 to 59 months in both zones is shown in Table 3. Whether it's in the treated zone or not, the IP is higher in Avagbodji's villages than in villages of Houédomè and Zoungamè. The global IP was lower in villages where the PSP have been installed compared to unprotected villages (16.62% *vs*. 72.20%). The analysis of Table 3 clarifies that the global IP is more than 4 times lower in the treated zone than in the control zone among children ($\chi^2 = 81.39$; $P<10^{-6}$) Figure 1.

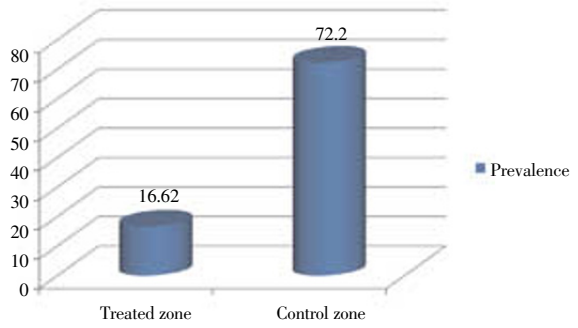

Figure 1. Global plasmodial prevalence of treated zone and control zone.

The plasmodial prevalence in both zones is low in the age groups (6–11) and (12–17) but too high in the control zone. The prevalence is high in the age group (18–23) and stabilizes from the age group (36–47). Moreover, the prevalence is generally low in the treated zone than in the control zone (Figure 2).

Table 3
Global plasmodic index per village in the control zone and in the treated zone among children aged 6 to 59 months.

Treated zone			Control zone		
Villages	Positifs (examined)	IP	Villages	Positifs (examined)	IP
Djèkpé	13 (93)	13.97%	Gbodjè	50 (63)	79.36%
Akpadon	20 (84)	23.80%	Bèmbè 1	62 (82)	75.60%
Agbodjèdo	10 (76)	13.15%	Akodji	56 (81)	69.13%
Donoukpa	7 (67)	10.44%	Trankomè	55 (85)	64.70%
Total	50 (320)	16.62%	Total	223 (311)	72.20%

$X^2 = 81.39$; $P<10^{-6}$.

Table 4
Parasites Density in each village of the control zone and the treated zone among children aged 6 to 59 months.

DP			
Control zone		Treated zone	
villages	M.G.	villages	M.G.
Djèkpé	700	Gbodjè	328
Akpadon	764	Bèmbè 1	448
Agbodjèdo	441	Akodji	221
Donoukpa	495	Trankomè	239
M.G.T	600	M.G.T	309

$X^2 = 0.86$; $P= 0.35$; M.G. = Geometric Mean; M.G.T. = Total Geometric Mean.

Table 5
Arithmetic mean of hemoglobin level in the control zone and in the treated zone among children aged 6 to 59 months.

Villages	Control zone			Treated zone	
	Examined	THb (IC à 95%)	Villages	Examined	THb (IC à 95%)
Djèkpé	77	8.8 (8.5–9.2)	Gbodjè	78	9.4 (9.0–9.8)
Akpadon	78	8.8 (8.5–9.2)	Bèmbè 1	81	9.8 (9.5–10.2)
Agbodjèdo	79	8.6 (8.2–9.)	Akodji	79	9.6 (9.2–10.0)
Donoukpa	77	8.6 (8.3–8.9)	Trankomè	82	9.3 (8.8–9.8)
Total	311	8.7	Total	320	9.5

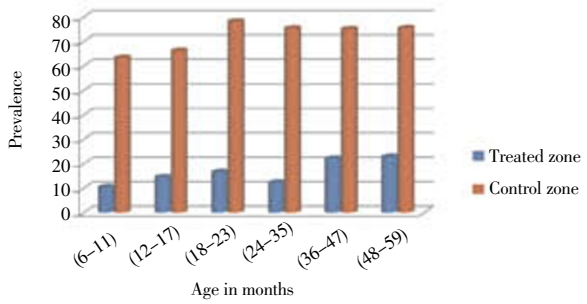

Figure 2. Plasmodial prevalence according to age groups in the control zone and in the treated zone.

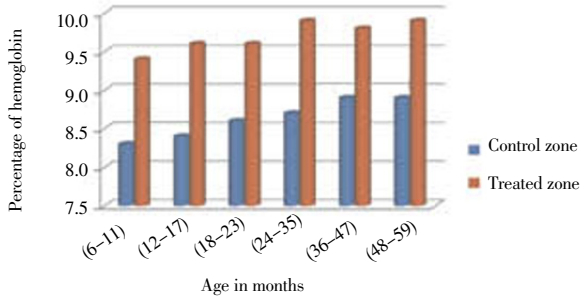

Figure 3. Haemoglobin level according to age.

3.2.2. Impact of PSP on gametocyte index to P. falciparum

Concerning the impact of PSP on this parameter, the zone analysis shows that the gametocyte index did not differ significantly between the treated zone and control zone (27.8% *vs*. 29.1%, $\chi^2 = 1.6$, $P = 0.18$). *P. falciparum* gametocytes have been encountered with a frequency of 35.67% for all zones (225 of 631 blades).

3.2.3. Impact of PSP on parasite density (DP)

The impact of PSP on DP, was deeply affected in all villages of the treated zone where we note, as shown in Table 4, the DP was two times lower in villages of treated zone than in the control villages (309 *vs*. 600 trophozoites of *P. falciparum* (tf/ μL of blood).

3.2.4. Impact of PSP on the prevalence of DP 5 000 tf/ μL of blood

Overall, 239 (37.8%) of 631 children had a parasitemia higher than or equal to 5 000 tf/ μL. The mean frequencies per zone were higher in the control zone, with a prevalence of 58.4% against 17.2% in the treated zone.

3.3. Impact of PSP on hemoglobin level (THb) and on prevalence of anemia

3.3.1. Impact of PSP on THb

The PSP has contributed to the significant increase THb in children living in the treated zone (9.5 g/dL) compared to children in the control zone (8.7 g/dL). The distribution of THb average values by zone is represented in Table 5 which indicates that they are greater than 9 g/dL in the treated zone while they are less than 9 g/dL in the control zone.

Age is a variable positively influencing the THb and Figure 3 clarifies that, whatever the treatment, the THb increases in children over 17 months. About the impact of PSP on THb based on age groups, a significant effect was observed in children over 24 months, among whom are noted THb higher in the treated area (9.8 g/dL) compared to the control zone (9.0 g/dL; $\chi^2 = 5.4$, P=0.001).

3.3.2. Prevalence of anemia

The prevalence of anemia (moderate and severe) was not significantly different between the treated zone and control zone. However, we noted a prevalence of anemia in the control zone compared to the treated zone (Table 6).

Considering the median of THb in the study population (9.2 g/dL), with 80% of the population ranges from 7.6 g/dL and 10.6 g/dL, we realize that practically all children are included among the moderate anemia (92% in control zone and 87% in the treated zone). By against, severe anemias were rare in our sample and, although they presented a significant difference 27% between the treated area (5.1%) and control area (6.9%), we could not demonstrate a difference significant. However, although few in our sample, children with mild hemoglobin (THb≥11 g/dL) were relatively more frequent in the treated zone (10%) than in the control zone (1.9%, $\chi^2 = 2.8$, P=0.0009).

Table 6
Crude prevalence of anemia according to the treatment groups among children aged 6–59 months.

Zones	Prevalence (positifs/examined)		
	Mild hemoglobin	moderate anemia	severe anemia
Control zone	6/311	287/311	22/311
Treated zone	32/320	279/320	16/320

3.4. Impact of the PSP on the prevalence of fevers

By comparing the prevalences of fever, we observe a difference between the control region (150 children on 311, 48.23%) and treated area (56 on 320, 17.5%). ($\chi^2 = 33.80$; $P=10^{-4}$). The risk of fever was significantly higher in children whose DP equalized or exceeded the value of 5 000 tf/ μL, compared to uninfected children. According to our calculations, it is from this level that the proportion of fevers attributable to malaria becomes appreciable. We found so interesting to measure the impact of PSP on cases of fever associated with this level of parasitemia. Against all expectation, considering the impact of PSP on DP 5 000≥ tf/ μL, the prevalence of fever cases with DP≥5 000 tf/ μL remained very similar between the two treatment groups

(7.5% for control zone and 6.9% treated zone ; $\chi^2 = 0.26$, $P = 0.80$).

4. Discussion

In Sub Saharan Africa, malaria is a leading cause of morbidity and mortality in children[14]. However, malaria infection itself is usually asymptomatic. This justifies the fact that the plasmodic index of the study was 72.20% in the control zone, while severe anemia in this area was 6.9%. One transversal study to determine the plasmodic index in an area, gave over 50% of school children, have positive thick smears without any associated symptoms[15,16]; parasite densities are then usually low. Among non immune patients that are infants, and in the case of *P. falciparum* only, the clinical condition can quickly deteriorate to evolve to severe anemia. In areas of stable malaria, the child will gradually acquire a protective immunity that can protect him against severe manifestations of malaria. The PSP has therefore performed a decisive impact in reducing anemia in the treated zone. In tropical Africa the gene for hemoglobin S (HbS) confers protection against malaria. The high frequency of this gene in sub-Saharan populations could result from a balance between on the one hand early mortality that drive the clinical manifestations of sickle cell anemia patients homozygote (SS) and, secondly, about the protection of uncomplicated and severe malaria, 60% and 90%[17] conferred by the gene heterozygotes (AS). This protection, which is a slower growth of the parasite in red blood cells containing HbS, is not complete and is expressed mainly in young children[18]. This could confirm the low prevalences in the two study zones among children of both age groups[6-11] and[12-17]. Hill's work in 1991 showed that the presence of HLA-Bw 53, frequently found in West Africa, caused a 40% decrease in the number of neuropaludismes and severe anemia[19]. The protection associated with major histocompatibility complex would be explained by an increase in the humoral response and the stimulation of cytotoxic T lymphocytes acting against the intrahépatocytaire stage of the parasite[20]. McGuire *et al*., in 1994, revealed the protective role of the developer gene of TNF against malaria pernicious attacks[21]. All children have an inborn protective factor of physiological origin. Indeed, the HbF inhibits the growth of *P. falciparum* in red blood cells[22]. This would explain, in part, the low parasite densities encountered during infections in young children in the study. This protection disappears gradually with the replacement of HbF by hemoglobin A. Hence the rise in prevalence in both studies zone from the age group[18-23]. Other factors related to the lifestyle of the child will have a role in the occurrence of malaria infections. Breast feeding, usually exclusive in Africa during the first months of life protects children. Indeed, the absence of para-aminobenzoic acid (PABA) in breast milk, limit parasite development that needs for the synthesis of its DNA[23]. In stable endemic zone, a large proportion of children with parasites are asymptomatic. The use of new methods such as PCR, including the detection threshold is lower than the thick film which is the gold standard, shows that the number of asymptomatic children is still underestimated[24]. In these areas where subjects gradually develop acquired immunity, the mere presence of parasites in the blood of a subject does not diagnose malaria. Thus, in one study, only 45% of subjects consulting for fever which had been made the diagnosis of malaria attacks indeed suffer from this disease[25]. Hence the need for determining the detection threshold. Several teams have calculated thresholds[16,26,27]. However, the confidence interval of these thresholds is wide and its choice will depend on the sensitivity and specificity desired. Rooth and Bjorkman, for the diagnosis of malaria, recommends a threshold of 400 parasites/μL for children older than one year, for a sensitivity of 96%, and no threshold for children younger than one year[28]. In a study conducted of children aged under 6 years in highly endemic area, Smith *et al*. find, for a threshold of 5 000 parasites/μL, sensitivity and specificity of 80%[16]. The pyrogenic threshold of parasitaemia is a good tool for epidemiological assessment but difficult to use in the interests of clinical diagnosis[29]. Different clinical algorithms have been proposed to refine the diagnosis of malaria as the duration and intensity of fever[30] or absence of cough, spleen size, presence of fever, vomiting, stool normal, the absence of pulmonary symptoms[31]. All bring improvements in terms of specificity for loss of sensitivity. However, many observations can be made, concerning in particular on the experimental conditions for the assessment of these algorithms. These misgivings are shared by Petersen *et al* who report observations of a subgroup of children who had lower body temperatures despite high parasite densities and suggest that this clinical condition could be an indicator of the occurrence of severe malaria among children[32]. Only 1% to 2% of children with malaria will develop a severe attack. The peak incidence of cerebral malaria in area of holo and hyperendemic malaria occurs around 45 months while severe anemia occur earlier to 28 months[33]. This explains the prevalence of our study which amount to 74% in age groups[24-35] and[48-59]. With PSP, the prevalences are reduced to 10% in age group[24-35] and 20% in age group[48-59]. Multiple hypotheses have been advanced to explain the causes of a shift to a severe attack[34]. The initial inoculum, possibly caused by several anopheles bites, could shorten the time to onset of heavy

parasitemia, time becomes too short to allow the child to develop an appropriate immune response and sufficient to control the multiplication of parasites. This would explain the effectiveness of treated nets which, without allowing a significant reduction in morbidity, would have an impact on mortality[35]. The virulence of strains, which may be variable, may also be mentioned[36]. This virulence may be expressed through the ability of the strain to secrete TNF by the cells. The latter is involved in the physiopathology of cerebral malaria. Mendis and Carter suggest the hypothesis that sensitization to the parasite may predispose the individual to the occurrence of a severe attack[37-46]. The production of interferon by activated potentiate cells enabling monocytes to produce TNF. Drug resistance strains could also affect the risk of progression to severe malaria. The child often happens, with or without treatment, to control malaria attacks occurring. However, these recurrent infections are not without consequences on the overall health and ability to fight against various diseases and environmental stresses to which children are subjected. Children anemias in Africa have two main sources: a nutritional origin with microcytic anemia caused by iron deficiency and anemia macrocytic caused by vitamin B_{12} or folate[47] and with intestinal parasitoses and malaria[48,49]. The prevalence of anemia varies according to study areas but is generally high. They can affect more than half of children such as Tanzania, where Premji et al report a prevalence of 74% in children younger than 3 years[50]. By protecting for one year a group of children using ITNs, Shiff *et al* noted a reduction by half of the prevalence of anemia compared to a group of control children[51]. Despite the increase in malaria prevalence and parasite densities from the age of four months[52], the prevalence of anemia decreased with age[51] suggesting that the risk factors of occurrence of anemia have changed and/or the child has a better capacity for regeneration of destroyed red blood cells[53]. This explains the gradual rise in hemoglobin in both study areas with increased rates of the treated area.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was supported by the Ministry of Higher Education and Scientific Research of the Government of Benin. I am also grateful to the medical laboratory's team of the hospital of Aguégués and the dressmakers of the commune. We thank the heads of households for their participation in the study. We also thank the children of the commune of Aguégués.

References

[1] Singh B, Kim Sung L, Matusop A. Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet* 2004; **363**(9414): 1017-1024.

[2] Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired *Plasmodium knowlesi* malaria in humans, Thailand. *Emerg Infect Dis* 2004; **10**(12): 2211-2213.

[3] Slinger R, Giulivi A, Bodie-Collins M, Hindieh F, John RS, Sher G, et al. Transfusion-transmitted malaria in Canada. *CMAJ* 2001; **164**(3): 377-379.

[4] Davies HD, Keystone J, Lester ML, Gold R. Congenital malaria in infants of asymptomatic women. *CMAJ* 1992; **146**(10): 1755-1756.

[5] World Health Organization. *World Malaria Report 2005*. Geneva: WHO and UNICEF; 2005.

[6] Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005; **434**(7030): 214-217.

[7] Choi HW, Breman JG, Teutsch SM, Liu S, Hightower AW, Sexton JD. The effectiveness of insecticideimpregnated bed nets in reducing cases of malaria infection: a meta-analysis of published results. *Am J Trop Med Hyg* 1995; **52**(5): 377-382.

[8] Fradin MS. Mosquitoes and mosquito repellents: a clinician's guide. *Ann Intern Med* 1998; **128**(11): 931-940.

[9] Lengeler C, Cattani J, Savigny D. *Net Gain: A New Method for Preventing Malaria Deaths*. Geneva and Ottawa: IDRC; 1996, p. 189.

[10] World Health Organization. *Report of the 11th WHOPES Working Group meeting; 2007 Dec 10-13*. Geneva Switzerland: World Health Organization; 2008.

[11] Smith T, Schellenberg AJ, Hayes R. Attributable fraction estimates and case definitions for malaria in endemic areas. *Stat Med* 1994; **13**: 2345-2358.

[12] WHO/USAID. *Les défis de la lutte contre le paludisme en Afrique*. Geneva: World Health Organization; 1994, p. 65.

[13] WHO. *Report of the African Regional consultation on control of anemia in pregnancy World Health Organization mimeographed document AFR/MCHI86*. Geneva: WHO; 1989.

[14] Bursaux E. La recherche antipaludéenne: un parent très pauvre de la recherche biomédicale. *Med Sci* 1997; **13**: 683-684.

[15] Trape JF, Greenwood BM. Approches nouvelles en épidémiologie du paludisme. *Ann Institut Pasteur/Actualites* 1994; **5**: 259-269.

[16] Smith T, Charlwood JD, Kihonda J, Mwankusye S, Billingsley P, Meuwissen P, et al. Absence of seasonal variation in malaria parasitaemia in an area of intense seasonal transmission. *Acta Trop* 1993; **54**: 55-72.

[17] Hill AV. Malaria resistance genes: a natural selection. *Trans R Soc Trop Med Hyg* 1992; **86**: 225-226.

[18] LE Hesran JY, Personne I, Personne P, Fievet N, Dubois B, Beyemé M, et al. Longitudinal study of *Plasmodium falciparum* infection and immune responses in infants with or without the sickle cell trait. *Int J Epidemiol* 1999; **28**: 793-798.

[19] Hill AV, Allsopp CE, Kwiatkowski D, Anstey NM, Twumasi P, Rowe PA, et al. Common west African HLA antigens associated with protection from severe malaria. *Nature* 1991; **352**: 595-600.

[20]Hill AV, Elvin J, Willis AC, Aidoo M, Allsopp CE, Gotch FM, et al. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. *Nature* 1992; **360**: 434-439.

[21]Mc-Guire W, Hill AV, Allsopp CEM, Grenwood BM, Kwjatkowski D. Variation in the TNF α promoter region associated with susceptibility to cerebral malaria. *Nature* 1994; **371**: 508-511.

[22]Pasvol G, Weatherall DJ, Wilson RJM. Effects of foetal haemoglobin on susceptibility of red cells to *Plasmodium falciparum*. *Nature* 1977; **270**: 171-173.

[23]Maegraith BG, Deegan T, Sherwood Jones E. Suppression of malaria, *Plasmodium berghei*, by milk. *Br Med J* 1952; **2**: 1382-1384.

[24]Bottius E, Guanzirolli A, Trape JF, Rogier C, Konate L, Druilhe P. Malaria: even more chronic in nature than previously thought ; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. *Trans R Soc Trop Med Hyg* 1996; **90**: 15-19.

[25]WHO. Meeting pathologie malaria diagnosis: memorandum from a WHO Meeting. *Bull WHO* 1988; **66**: 575-594.

[26]Trape JF, Peelman P, Morault-Peelman B. Criteria for diagnosing clinical malaria among a semi-immune population exposed to intense and perennial transmission. *Trans R Soc Trop Med Hyg* 1985; **79**: 435-442.

[27]Rogier C, Trape JF. Etude de l'acquisition de la prémunition en zones d'holo et de méso-endémie palustre à Dielmo et à Ndiop (Sénégal) : résultats préliminaires 1990-1994. *Med Trop* 1995; **55**(Suppl 4): 71S-76S.

[28]Rooth I, Bjorkman A. Fever episodes in a holoendemic malaria area of Tanzania: parasitological and clinical findings and diagnostic aspects related to malaria. *Trans R Soc Trop Med Hyg* 1992; **86**: 479-482.

[29]Gendrel D, Kombila M, Martz M, Nardou M, Lecointre C, Gendrel C, et al. Parasitémie au cours des accès palustres à *Plasmodium falciparum* chez l'enfant. *Presse Med* 1992; **21**: 1805-1808.

[30]Rougemont A, Breslow N, Brenner E, Moret AL, Dumbo O, Dolo A, et al. Epidemiological basis for clinical diagnosis of childhood malaria in endemic zone in West Africa. *Lancet* 1991; **338**: 1292-1295.

[31]Genton B, Smith T, Baea N. Malaria: how useful are clinical criteria for improving the diagnosis in a highly endemic area? *Trans R Soc Trop Med Hyg* 1994; **88**: 537-541.

[32]Petersen E, Hogh B, Marbiah NT. Development of immunity against *Plasmodium falciparum* malaria: clinical and parasitologic immunity can not be separated. *J Infect Dis* 1991; **164**: 949-953.

[33]Brewster DR, Kwiatkowski D, White NJ. Neurological sequelae of cerebral malaria inchildren. *Lancet* 1990; **336**: 1039-1043.

[34]Greenwood BM, Marsh K, Snow RW. Why do some African children develop severe malaria? *Parasitol Today* 1991; **7**: 277-281.

[35]Alonso PL, Lindsay SW, Armstrong JR, Konteh M, Hill AG, David PH, et al. The effect of insecticide-treated bed nets on mortality of Gambian children. *Lancet* 1991; **337**: 1499-1502.

[36]Gupta S, Hill AV, Kwiatkowski D, Greenwood BM, Greenwood AM. Parasite virulence and disease patterns in *Plasmodium falciparum* malaria. *Proc Natl Acad Sci USA* 1994; **91**: 3715-3719.

[37]Mendis KN, Carter R. Clinical disease and pathogenesis in malaria. *Parasitol Today* 1995; **11**: 1-16.

[38]Osonuga OA, Osonuga A, Osonuga AA, Osonuga IO. Resolution pattern of jaundice among children presenting with severe malaria in rural South-West Nigeria. *Asian Pac J Trop Biomed* 2012; **2**(7): 551-553.

[39]Reza YM, Taghi RM. Prevalence of malaria infection in Sarbaz, Sistan and Bluchistan province. *Asian Pac J Trop Biomed* 2011; **1**(6): 491-492.

[40]Zerihun T, Degarege A, Erko B. Association of ABO blood group and *Plasmodium falciparum* malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pac J Trop Biomed* 2011; **1**(4): 289-294.

[41]Viroj W. Concurrent malaria and dengue infection: a brief summary and comment. *Asian Pac J Trop Biomed* 2011; **1**(4): 326-327.

[42]Krungkrai SR, Krungkrai J. Malaria parasite carbonic anhydrase: inhibition of aromatic/heterocyclic sulfonamides and its therapeutic potential. *Asian Pac J Trop Biomed* 2011; **1**(3): 233-242.

[43]Lorenz V, Karanis P. Malaria vaccines: looking back and lessons learnt. *Asian Pac J Trop Biomed* 2011; **1**(1): 74-78.

[44]Mohammad A, Mansoreh S, Mehdi K, Hasan V, Reza AM, Kamran A. Persistence and residue activity of deltamethrin on indoor residual spraying surfaces against malaria vectors in southeastern Iran. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S271-S275.

[45]Shetty G, Avabratha KS, Gonsalves S, Dany A, Rai BS. Thrombocytopenia in children with malaria-A study from coastal Karnataka, India. *Asian Pac J Trop Dis* 2012; **2**(2): 107-109.

[46]Alaya-Bouafif NB, Chahed MK, Bez HE, Bellali H, Ayari L, Achour N. Completeness of malaria notification in Tunisia assessed by capture recapture method. *Asian Pac J Trop Dis* 2012; **2**(2): 187-191.

[47]Dallman PR, Siimes MA, Stekel A. Iron deficiency in infancy and childhood. *Am J Clin Nutr* 1980; **33**: 86-118.

[48]Hedberg K, Shaffer N, Davachi F, Hightower A, Lyamba B, Paluku KM. *Plasmodium falciparum*-associated anemia in children at a large urban hospital in Zaïre. *Am J Trop Med Hyg* 1993; **48**: 365-371.

[49]Greenwood BM, Bradley AK, Greenwood AM, Byass P, Jammeh K, Marsh K. Mortality and morbidity from malaria among children in a rural area of the Gambia, West Africa. *Trans R Soc Trop Med Hyg* 1987; **81**: 478-486.

[50]Premji Z, Hamisi Y, Shiff C, Minjas J, Lubega P, Makwaya C. Anaemia and *Plasmodium falciparum* infections among young children in an holoendemic area, Bagamoyo, Tanzania. *Acta Trop* 1995; **59**: 55-64.

[51]Shiff C, Checkley W, Winch P, Premji Z, Minjas J, Lubega P. Changes in weight gain and anaemia attributable to malaria in Tanzanian children living under holoendemic conditions. *Trans R Soc Trop Med Hyg* 1996; **90**: 262-265.

[52]Kitua AY, Smith T, Alonso PL, Masanja H, Urassa H, Menendez C, et al. *Plasmodium falciparum* malaria in the first year of life in an area of intense and perennial transmission. *Trop Med Int Health* 1996; **1**: 475-484.

[53]Cornet M, LE Hesran JY, Fievet N, Cot M, Personne P, Gounoue R, et al. Prevalence of and risk factors for anemia in young children in Southern Cameroon. *Am J Trop Med Hyg* 1998; **58**: 606-611.